# Post translationally modified homocitrulline induced by MDSCs can be an effective anti-tumour target for CD4 T cells

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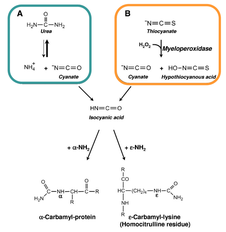
## INTRODUCTION

- Targeting post translationally modified epitopes may provide new targets for cancer vaccines that avoid the attenuation often seen with responses to self antigens[1, 2]
- Carbamylation of lysine residues leads to homocitrulline (Hcit), converting the positively charged amino acid to a neutral amino acid which may lead to a new epitope that is recognised by T-cells and B-cells
- During inflammation, carbamylation is driven by myeloperoxidase (MPO) an enzyme produced by neutrophils, macrophages and myeloid derived suppressor cells (MDSCs)[3]
- Two proteins that are subject to carbamylated are the cytoskeletal protein vimentin and the glycolytic protein aldolase, both of which are upregulated in some cancer cells
- In this study we demonstrate that Hcit specific CD4 responses can be generated and that these have an anti-tumour effect which is dependent on MDSC populations and MHC II presentation on the tumour

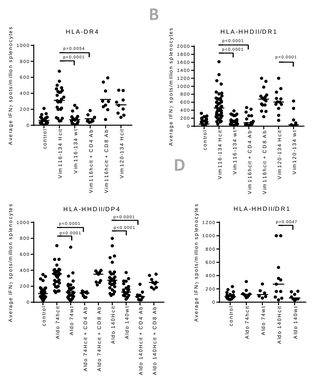



**Carbamylation. A modification that is driven by increased isocyanic acid levels. This occurs physiologically due to the breakdown of Urea (A) or the actions of MPO enzyme on thiocyanate (B). Carbamylation converts positively charged lysine to neutrally charged homocitrulline.[4]**

## Homocitrullinated peptides stimulate strong CD4 responses *in vivo*

- Studies were carried out using transgenic mice expressing different human HLA types
- Mice were immunised with Hcit peptides with adjuvant CpG/MPLA
- IFNγ responses were then detected by ELISpot
- HLA-specific responses were observed to the three peptides used

| | HHDII/DP4 | HHDII/DR1 | DR4 |
|---|---|---|---|
| Aldolase 74Hcit | ✓ | ✗ | ✗ |
| Aldolase 140Hcit | ✓ | ✓ | ✗ |
| Vimentin 116Hcit | ✗ | ✓ | ✓ |

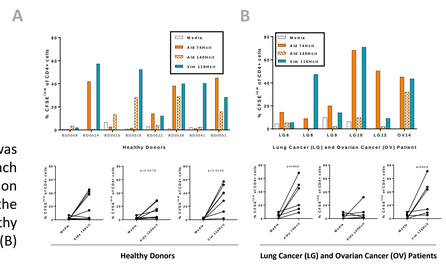


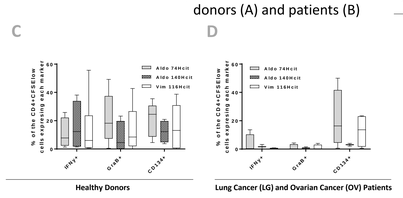

***In vivo* responses**. Immunisation with Hcit Vimentin peptides induced responses in HLA-DR4 (A) and HLA-HHDII/DR1 mice (B). Immunisation with Hcit Aldolase peptides induced responses in HLA-HHDII/DP4 (C) and HLA-HHDII/DR1 mice (D). Responses were Hcit specific and CD4 mediated for all three peptides

## Humans have a repertoire for homocitrullinated peptides

- PBMCs were isolated from 8 healthy donors and 6 cancer patients.
- PBMCs were CD25 depleted and CFSE labelled then stimulated with each peptide for 10 days

**Proliferative responses**. Proliferation was measured in the stimulated samples from each patient. Most donors showed CD4 proliferation in response to at least one of the Homocitrullinated peptides in both the healthy donors (A) and patients (B)

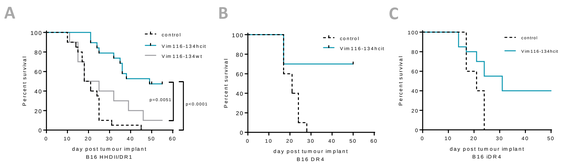



**Cytokine responses**. Donors that responded to each peptide were assessed for other markers. The % of $CD4^+CFSE^{low}$ cells expressing each marker is shown for healthy donors (C) and Cancer patient (D) responders

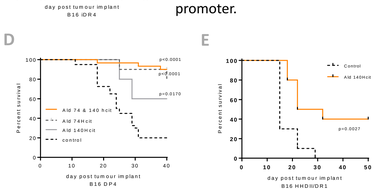
## Homocitrullinated peptides stimulate an anti-tumour responses *in vivo*

- Mice were implanted with B16F1 tumour cells transfected with human HLA on day 1. Treated mice were then immunised with Hcit peptides on day 4, 11 and 18.
- Tumour growth and survival was monitored



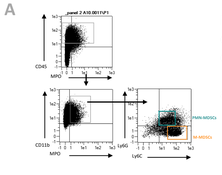

**Vimentin Hcit survival**. Increased survival was seen for HHDII/DR1 (A) and DR4 (B) mice immunised with the Vimentin Hcit peptide. The response was also observed in B16 iDR4 (C) cells where the DR4 is under an IFNγ-inducible promoter.

**Aldolase Hcit survival**. Increased survival when compared to control was seen for HHDII/DP4 (D) and HHDII/DR1 (E) mice immunised with Aldolase Hcit peptides. Aldolase 74Hcit and Aldolase 140Hcit peptides were also combined in HHDII/DP4 mice and showed a good anti-tumour response.



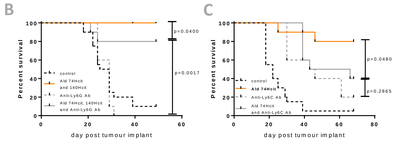

## MDSCs and MHCII presentation are required for target recognition and survival

- B16F1 cells were grown *in vivo* and then dissected, disaggregated and stained for MDSC markers

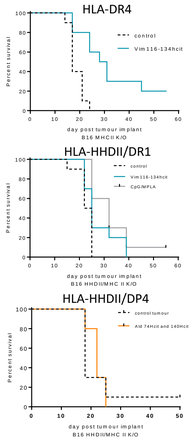



**MPO staining**. Tumour infiltrating lymphocytes were stained for MPO enzyme and immune cell markers. MPO+ cells were CD45+ CD11b+ and either Ly6G+Ly6C$^{low}$, markers associated with a population of polymorphonuclear MDSCs (PMN-MDSCs) or Ly6G-Ly6C$^{hi}$, markers associated with monocytic MDSCS (M-MDSCs). In the tumour both populations included MPO+ cells.

- Anti-tumour studies were carried out using Aldolase Hcit peptides in the presence of antibodies which depleted either the Ly6G+ or Ly6C+ populations




**Removal of Ly6G or Ly6C population on survival**. Mice immunised with Aldolase Hcit peptides in the presence of antiLy6G antibody showed little loss of survival (B). In contrast mice immunised with Aldolase Hcit peptides in the presence of anti-Ly6C antibodies showed reduced survival compared to Hcit peptides only (C).




- Mice were implanted with B16 F1 cells without MHC II
- Immunised mice were then given three doses of Hcit peptides
- Tumour growth and survival were monitored

**Survival in MHCII deficient model**. Immunisation with Vim Hcit peptide had minimal effect on survival in DR4 (D) or HHDII/DR1 (E) mice challenged B16F1 cells without MHC II expression.
Similarly immunisation with Aldolase Hcit peptides had no effect on survival in HHDII/DP4 mice (F) challenged with B16F1 cells without MHC II expression.

## CONCLUSIONS

- **Homocitrullinated Vimentin and Aldolase peptides induce CD4 T-cells responses in a number of HLA types**
- **Responses after Hcit peptide vaccination do not cross react with wild type, non-homocitrullinated peptides and are CD4 mediated**
- **Human donors also showed a repertoire of CD4 T-cells that respond to these peptides**

### Immunisation with Homocitrullinated peptides induces strong CD4 responses

- **Hcit T-cells responses are associated with increased survival in mice challenged with B16F1 cells expressing MHCII**
- **Increased survival suggests that tumour cells undergo carbamylation perhaps due to increased MPO expression**
- **Responses are attenuated in mice which have been depleted of Ly6C$^{high}$ cells which includes MPO producing MDSCs**
- **However, responses also require tumour cells to have the ability to express MHC II**

### Hcit peptide responses lead to increased survival *in vivo*
### This depends on MPO production by MDSCs leading to increased carbamylation in the tumour microenvironment and MHCII presentation of homocitrullinated epitopes

## References:

1. Brentville VA, Metheringham RL, Gunn B, Symonds P, Daniels I, Gijon M, Cook K, Xue W, Durrant LG (2016). Citrullinated vimentin presented on MHC-II in tumor cells is a target for CD4+ T cell-mediated antitumor immunity. Cancer Research 2016 Feb 1;76(3):548-60
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3. Holzer, M., K. Zangger, D. El-Gamal, V. Binder, S. Curcic, V. Konya, R. Schuligoi, A. Heinemann, and G. Marsche. 2012. 'Myeloperoxidase-derived chlorinating species induce protein carbamylation through decomposition of thiocyanate and urea: novel pathways generating dysfunctional high-density lipoprotein', Antioxid Redox Signal, 17: 1043-52.
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